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Soil-Inhabiting Fungi Attacking the Roots of Maize

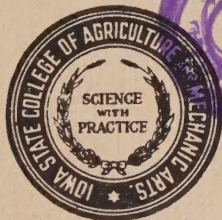
BY WEN-CHUN HO

AGRICULTURAL EXPERIMENT STATION
IOWA STATE COLLEGE OF AGRICULTURE AND
MECHANIC ARTS

BOTANY AND PLANT PATHOLOGY SECTION

BUREAU OF PLANT INDUSTRY, SOILS AND AGRICULTURAL ENGINEERING
UNITED STATES DEPARTMENT OF AGRICULTURE

Cooperating



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SUMMARY

During the course of the present study various organisms were found to be associated with decayed seeds and infected roots, mesocotyls, plumules and crowns of maize, both in the greenhouse and in the field. The organisms found most often on the decayed seeds were *Pythium debaryanum*, *Gibberella saubinetii*, *Penicillium oxalicum*, *Trichoderma lignorum* and *Fusarium* spp. Those occurring on diseased roots were *Pythium debaryanum*, *P. graminicola*, *Gibberella saubinetii*, *Helminthosporium sativum*, *Rhizoctonia solani*, *Trichoderma lignorum*, *Fusarium moniliforme* and *Fusarium* spp. Isolations made from infected mesocotyls yielded chiefly *Gibberella saubinetii*, *Rhizoctonia solani*, *Helminthosporium sativum*, *Penicillium oxalicum*, *Trichoderma lignorum* and *Fusarium* spp., and those from diseased plumules yielded *Gibberella saubinetii*, *Fusarium* spp. and *Rhizopus* sp. The organisms that appeared on the crowns were *Diplodia zeae* and *Fusarium moniliforme*.

These organisms may be divided into three groups based on their pathogenicity on maize in the greenhouse and under field conditions: (a) highly destructive, *Pythium debaryanum*, *Pythium graminicola* and *Gibberella saubinetii*; (b) moderately destructive, *Rhizoctonia solani*, *Helminthosporium sativum*, *Diplodia zeae* and *Penicillium oxalicum*; (c) slightly destructive, *Aspergillus niger*, *Fusarium moniliforme*, *Trichoderma lignorum*, *Rhizopus* sp. and *Fusarium* spp.

Greenhouse and field data indicated that *Pythium debaryanum* was responsible for heavy seed decay of maize, particularly when the soil temperature was below 16° C. and moisture was abundant. *Gibberella saubinetii* and *Pythium graminicola* were also capable of causing considerable decay in cases where *P. debaryanum* was scarce or inactive in the soil. Initial root infection of maize was caused by *P. debaryanum* and *P. graminicola*. This injury was typified by large, brownish, water-soaked necrotic lesions on the terminal portions of the roots followed by the destruction of many or all of the fine rootlets. *Gibberella saubinetii* often caused reddish-brown to dark red necrotic lesions on the remaining portions of the roots but more characteristically produced a reddish-brown discoloration and rot of the mesocotyl. Most strains of *Helminthosporium sativum* were only slight-

ly pathogenic, but one strain, isolate 9035, repeatedly produced a brownish-black discoloration and rot of the mesocotyls and roots, thus causing a severe stunting and blighting of seedlings in the greenhouse. Under field conditions these symptoms often were masked by the symptoms previously produced by more aggressive pathogens. The other organisms heretofore mentioned, with the exception of one strain of *Rhizoctonia solani*, were found to be either secondary invaders or saprophytes.

In general, the combination of two active pathogens, such as *Pythium debaryanum* with *Gibberella saubinetii*, seemed to increase the disease severity. Combinations of an active pathogen with a nonpathogenic or saprophytic form, e. g. *Gibberella saubinetii* with *Aspergillus niger* or with *Penicillium oxalicum*, sometimes decreased the severity of the injury as compared with that produced by the active pathogen alone.

During the last half of May, *Pythium debaryanum* was found to become parasitic before and following emergence, causing seed decay, stunted growth and tip necrosis on the primary and some of the seminal roots. As the season advanced and the soil reached a higher temperature, *P. graminicola* became aggressive and induced a rapid necrosis with a brown, water-soaked appearance on the tips of most of the roots. At the same time *Gibberella saubinetii* and *Rhizoctonia solani* often occurred on the lower part of the mesocotyl and later caused a discoloration and necrosis of the roots. As the season advanced other organisms such as *Helminthosporium sativum* and *Penicillium oxalicum* produced further necrotic lesions on the mesocotyl and basal portion of the roots. Still later in the sequence, *Fusarium* spp., *Aspergillus niger*, *Trichoderma lignorum* and other saprophytic soil-inhabiting organisms were isolated from the root and mesocotyl lesions. This later group of organisms often developed extensively in the maize seedlings, leaving no evidence of the organisms that had prevailed earlier.

Soil-Inhabiting Fungi Attacking the Roots of Maize¹

By WEN-CHUN HO

Several pathogens were known to attack the roots of maize, but their effect was not well understood. These organisms seemed to attack not only singly but in combination with one another and in a certain succession, influenced by environmental conditions.

The present study was undertaken to investigate the relative importance of (a) various pathogens attacking seeds and seedlings in their early stages of development, (b) symptoms manifested by each pathogen singly and in combination, (c) the injuries produced by each pathogen under laboratory, greenhouse and field conditions, and (d) the influence of environmental factors upon the succession of pathogens on the seeds and seedlings.

MATERIALS AND METHODS

SOURCES OF SEEDS

Two inbreds, Lancaster 317 and Black 345, and one hybrid, Iowa 939, were employed in these studies. Lancaster 317 was used in many of the studies because of its moderate susceptibility to most of the root pathogens. Black 345 was chosen because of its extreme susceptibility to *Pythium*, and Iowa 939 because it was fairly resistant to *Pythium*. The viability of the seed was determined prior to the experimentation. All the seed lots used germinated 98 percent or better at favorable temperatures. To remove any surface infestation the seeds were soaked in distilled water for 1 to 2 hours, surface-disinfected with 10 percent commercial Clorox for 10 minutes, and then washed with steamed distilled water two or three times before planting.

ISOLATION

Isolations of soil-inhabiting plant pathogens from the decayed seeds and infected parts of roots, mesocotyls and crowns of maize have been made during the past 5 years. The diseased seeds and seedlings were dug in the field and

¹Taken from part of a thesis submitted to the faculty of the Graduate College, Iowa State College, in partial fulfillment of the requirements for the degree Doctor of Philosophy. Project 432 of the Iowa Agricultural Experiment Station, Ames, Iowa.

wrapped immediately in wet paper towels with a small quantity of soil. In the laboratory the material was soaked in tap water for a few hours and then washed with running tap water to remove the soil particles. The characteristic symptoms were recorded and the infected parts cut into fragments. These fragments were washed thoroughly in distilled water with a camel's-hair brush to remove the last trace of soil, then placed in sterile petri dishes containing steamed, distilled water which was changed three or four times at hourly intervals. Surplus water was removed by pressing the infected tissue between folds of sterile filter paper. To give most of the root parasites an opportunity to grow, several cultural media were used: Plain agar, potato-dextrose agar, malt agar and Czapek's sucrose-nitrate agar.

Two methods were applied for plating the infected tissue on medium. (a) Using Meredith's (43) method, a V-shaped slit was cut in the medium. This V-shaped portion was lifted with a flat, curved transferring needle, and a fragment of the infected tissue was placed under it. The needle was removed and the medium allowed to settle as nearly as possible into its former position. These cultures were incubated at 20° C. for 24 to 72 hours. The mycelium usually grew up through the medium onto the upper surface of the agar, from which hyphal tips were then transferred to agar slants, thereby avoiding the contaminants on the under side of the medium. The culture could be purified further by this same method. (b) A small piece of the medium was removed with a transferring needle. With a pair of forceps a fragment of infected tissue was placed underneath the medium through the hole, which was then covered with the removed piece of medium. Three fragments of infected tissue could be plated on the same petri dish. The hyphal tips of the pathogen were transferred to agar slants in the same manner as in the first method described above.

PREPARATION OF INOCULA

The inocula used in the greenhouse experiments were prepared by growing the organisms on plain agar plus steamed carrot and on potato-dextrose agar. In preparing inocula for the field experiments the pathogens were cultured on the following two types of media: (a) Corn meal-sand combination. This combination, consisting of 500 grams of corn meal and 1000 grams of washed white sand, was placed in glass jars and moistened with water, steamed for 30 minutes without pressure and autoclaved for two hours at 15 pounds pressure. (b) Oat giant culture. Oat

seeds were washed with tap water and then soaked in distilled water for 48 hours. More water was added and the medium steamed for 30 minutes without pressure and autoclaved for an hour at 15 pounds pressure.

LABORATORY AND GREENHOUSE TRIALS

The injuries induced on maize by certain pathogens and the effect of temperature on the production of injuries were studied under controlled conditions in high- and low-temperature, lighted chambers in the laboratory. No light was used for 2 in every 24 hours. One chamber was held at 15° C. and the other at 26° C. In each chamber 12 four-inch pots were employed for growing the seedlings. Six pots were filled with naturally infested field soil and six with steamed soil. The organism was allowed to grow in the soil for 2 to 3 days, and then 10 kernels were planted per pot. In the low-temperature chamber data were recorded 3 to 5 weeks after planting, and in the high-temperature chamber, 2 to 4 weeks after planting.

The greenhouse trials were conducted in two sets (A and B) of experiments. Each set consisted of three series and each series of three replications. Artificially infested steamed field soil and non-infested steamed field soil in five-inch pots were used for growing the seedlings. The soil was infested by introducing a mixture of steamed soil and the culture of a designated pathogen into the top 3 or 4 cm. of soil. Ten kernels were planted per pot. In experiments involving *Gibberella saubinetii* (Mont.) Sacc., *Helminthosporium sativum* P., K. and B. and *Fusarium moniliforme* Sheldon, the kernels were surface-disinfected and then soaked in a spore suspension of the respective pathogen for about 30 minutes.

Plants in set A were exposed continuously to greenhouse temperatures ranging from 20° to 28° C. Plants in set B were held at 9° C. for the first 10 days and then exposed to the greenhouse temperatures for the remainder of the experiment. The temperatures at which the two sets were held corresponded in part at least to the early and late field plantings. To reduce place effect the pots on the bench were randomized and changed once every week. The plants were removed from pots in set A after 4 or 5 weeks and from pots in set B after 5 or 6 weeks. Notes were taken on the roots and tops. The number of seedlings that survived at the end of the experiment also served as a criterion of infection.

FIELD TRIALS

Field trials for the study of the injuries induced by various pathogens were conducted at the North Iowa Agricultural Experimental Association Farm, Kanawha, from 1938 to 1940, at Ames from 1939 to 1940, and at the Southeastern Iowa Agricultural Experimental Association Farm at Conesville from 1939 to 1940. At Kanawha the experiments were conducted on soil that was mainly Webster silty loam having a pH 6.2 to 6.6. The crop on this land previous to 1938 was sugar beets. The soil of the experimental field at Ames was Clarion loam with a pH 6.4 to 6.7. Maize had been planted in this field in 1938 and 1937. At Conesville the experiments were located on sandy loam having a pH 4.5 to 5.2. The previous crops were maize in 1938 and oats in 1937.

The soil at Ames was partially disinfected with formaldehyde solution. At Kanawha and Conesville the soil was not disinfected except in the blocks that were infested with *Pythium debaryanum* Hesse and *Helminthosporium sativum*. These blocks were treated like those at Ames.

About 10 days after the disinfection, the soil was infested by introducing either a corn meal-sand combination or an oat giant culture of the pathogen at the rate of approximately 50 grams per row. Each experimental block was divided into two plots, and each plot was subdivided into five 5-foot rows. One plot was infested with the culture of a designated pathogen, and the other plot was maintained as a check. All the plots were randomized and replicated three times for each pathogen with the exception of *Pythium debaryanum*, in which case each plot was replicated four times.

About a week after infestation, seeds were planted at the rate of 50 kernels per row. Plantings were made at Conesville about April 15, at Ames about April 20 and at Kanawha around April 25. Stand counts usually were made 3 weeks after planting. Plants of the three central rows were measured 4 to 5 weeks after planting. The severity of the disease and the characteristic symptoms appearing on underground parts were recorded.

SYMPTOMS AND DESTRUCTIVENESS OF NINE
ORGANISMS ON THE SEEDS, ROOTS AND
MESOCOTYLS OF MAIZE

The underground parts of the maize plants examined in the field generally were parasitized by many organisms,

and the symptoms of the various pathogens could not readily be distinguished from one another. The first problem was to identify the organisms that might be pathogenic on seeds, roots and mesocotyls of maize in the soil and then to study the symptoms and injuries caused by each soil-inhabiting pathogen individually. Isolations were made from parasitized seeds and seedlings throughout the growing season, and the effect on maize seedlings of the organisms encountered most frequently was studied in detail. The symptoms produced by each organism under greenhouse conditions are summarized in table 19.

PYTHIUM DEBARYANUM HESSE

Pythium debaryanum was first reported on maize roots in 1874 by Hesse (20) from Germany. Speschneff (62) reported that *P. debaryanum* was observed on young maize in the Caucasus of Russia. In 1921 a dry rot (stalk rot) of maize caused by *P. debaryanum* was reported by Miura (45) from Manchuria. Eddins (12) in 1930 stated that *Pythium* root rot was one of the minor root diseases of maize in Florida. He found it severe on plants growing in cold, wet soil, whereas much less damage occurred in warm soil. In a recent study on succession of soil-inhabiting fungi attacking the roots of maize, Ho and Melhus (22) pointed out that *P. debaryanum* was one of the earliest soil pathogens attacking the seed and root tips of maize seedlings. They considered this organism to be the chief cause of seed decay and stunted growth of seedlings.

During the later part of May 1938, 1939 and 1940, *P. debaryanum* was isolated frequently from necrotic lesions on root tips of maize seedlings grown at Kanawha, Conesville and at Ames. This organism also occurred commonly on the decayed seeds in the field in May of each year from 1937 to 1940 inclusive, and evidently was responsible for poor stands in early plantings. Characteristic symptoms are illustrated in fig. 1.

To ascertain the type and extent of injury by *P. debaryanum* on maize, experiments were conducted in the laboratory, greenhouse and field. Seed of inbred Black 345 was used in all experiments.

The laboratory experiments were conducted with soil that was naturally infested with *P. debaryanum* and with steamed soil. The soil was a sandy loam having a pH 4.6 to 5.2 and was collected on the experimental plots at Conesville.

At 15° C., 60 to 100 percent of the seeds were decayed in

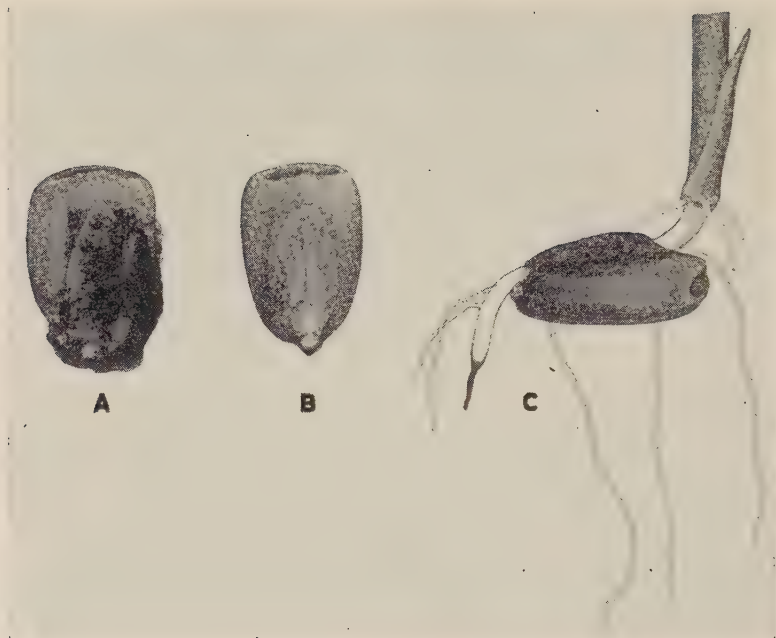


Fig. 1. Characteristic symptoms of *Pythium debaryanum* on maize seeds and seedling Black 345, grown in the greenhouse. A. Seed decayed and partially coated with a mass of mycelium and soil particles. B. Germinating seed with tip of radicle rotted. C. Seedling with primary root tip rotted and discolored pericarp.

the infested soil, but about 95 percent of the seeds in the check pots germinated. Sixty-one percent of the isolates obtained from these decayed seeds were *P. debaryanum*; the remaining 39 percent were organisms such as *Gibberella saubinetii*, *Fusarium moniliforme* and *Fusarium* spp. Small, brown, water-soaked necrotic lesions were found on the roots of the seedlings taken from the infested soil, but those taken from the check pots showed no trace of infection. The lesions appeared chiefly at the tips of the fine rootlets; however, they were observed sometimes on the larger seminal roots. *Pythium debaryanum* was isolated frequently from these necrotic lesions. Inoculation with these cultures induced the same type of root injury, and the pathogen was re-isolated from the infected tissue.

At 26° C., seed germination in the infested soil pots was 12 to 25 percent lower than in the check pots. There was, however, only a slight difference between length of roots of the seedlings taken from the infested soil and length of

those taken from the check pots. The roots of some of the seedlings taken from the infested soil showed a slight discoloration, but no distinct injury could be found.

The pathogenicity of *P. debaryanum* on maize was greatly affected by the temperature. A low soil temperature combined with 70 percent or more soil moisture apparently was most favorable for infection.

The data obtained from the greenhouse experiments are presented in table 1. Stand, top growth and root length of the seedlings were affected by the pathogen, particularly when the plants were held at 9° C. for the first 10 days, this temperature being favorable for the infection but unfavorable for the development of the seedling. The percentage decreases of stand count and of top growth in set B were twice as great as those in set A, and the decrease of root length in set B was five times that in set A.

Field experiments were conducted in 1938, 1939 and 1940 on the Kanawha experimental plots. The percentage decreases of stand observed in field tests during 3 years (table 2) were all over 60 percent. It should be noted that the stand trend was constantly lower in the infested than

TABLE 1. AVERAGE STAND, TOP GROWTH AND ROOT LENGTH (CM.) OF MAIZE SEEDLINGS GROWN FROM 10 SEEDS OF INBRED BLACK 345 IN SOIL INFESTED OR NOT INFESTED WITH *PYTHIUM DEBARYANUM* AT TWO TEMPERATURES IN GREENHOUSE EXPERIMENTS.

Temperature	20°-28° C. (A)				9° and 20°-28° C. (B)				Mean difference at two temperatures
Series	1	2	3	Mean	1	2	3	Mean	
Stand count									
Soil not infested.....	9.0	7.5	8.0	8.2	9.0	6.5	8.0	7.8	2.8
Soil infested.....	5.0	5.0	6.0	5.3	1.5	2.0	3.0	2.2	
Decrease.....	4.0	2.5	2.0	2.8	7.5	4.5	5.0	5.6	
Percentage decrease.....				34.7				72.4	
Value of t.....				4.7*				6.1*	6.5*
Top growth									
Soil not infested.....	21	24	23	22.6	18	20	22	20	4.7
Soil infested.....	18	20	17	18.3	8	10	15	11	
Decrease.....	3	4	6	4.3	10	10	7	9	
Percentage decrease.....				19.0				45.0	
Value of t.....				4.9*				9*	2.5
Root length									
Soil not infested.....	5.8	6.0	5.2	5.7	4.0	6.0	5.0	5.0	1.2
Soil infested.....	5.5	5.5	5.0	5.3	2.5	4.0	4.0	3.5	
Decrease.....	.3	.5	.2	.3	1.5	2.0	1.0	1.5	
Percentage decrease.....				5.8				30.0	
Value of t.....				3.7				5.2*	5.8*

* Between 5 percent and 1 percent levels.

TABLE 2. AVERAGE STAND FROM 50 SEEDS OF INBRED BLACK 345 IN SOIL INFESTED OR NOT INFESTED WITH *PYTHIUM DEBARYANUM* IN EARLY (LAST WEEK IN APRIL) FIELD PLANTINGS AT KANAWHA.

Year	1938					1939					1940					Mean 3 years
	1	2	3	4	Mean	1	2	3	4	Mean	1	2	3	4	Mean	
Soil not infested.....	30	29	30	31	30.0	35	40	36	41	38.0	30	28	32	27	29.3	32.4
Soil infested.....	9	6	12	8	8.8	10	15	17	13	13.8	9	11	10	7	9.3	10.6
Decrease.....	21	23	18	23	21.3	25	25	19	28	24.3	21	17	22	20	20.0	21.8
Percentage decrease.....					70.8					63.8					68.4	67.4
Value of t.....					18.0†					12.9†					18.5†	

† Beyond 1 percent level.

in the noninfested soil. Not only was there a sharp decrease in stand similar to that developing in the greenhouse experiments, but also much injury to the roots and aerial portions of the seedlings similar to that found in the greenhouse trials. *Pythium debaryanum* was readily isolated from the seeds and seedling roots.

PYTHIUM GRAMINICOLA SUBR.

Pythium graminicola probably was first reported on maize roots by Johann, Holbert and Dickson (30) in 1926, although they were unable to identify the species. In the same year, Valleau, Karraker and Johnson (69) claimed that maize root rot was caused by a soil-inhabiting pathogen, which they reported as an undetermined species of *Pythium*. A year later, a *Pythium*-like fungus was isolated from diseased maize roots in Missouri by Branstetter (2), who also did not name the species.

In 1928 Subramaniam (64) described a new species of *Pythium* on wheat affected with severe root rot in India. By cross inoculation on maize, he was able to produce infection. The fungus was named *P. graminicolum* Subr. The parasitism of *P. graminicola* on maize was further demonstrated by Johann, Holbert and Dickson (31). This same organism was studied by Drechsler (10) who later classified it as *Pythium arrhenomanes* Drech. Edgerton, Tims and Mills (13), while working with root rot of sugar cane in Louisiana, reported that a species of *Pythium* isolated from the infected roots of sugar cane was capable of attacking maize as readily as sugar cane.

In 1929 a new and serious disease of maize was studied by Curzi (5) in the vicinity of Pavia, Italy, where it resulted in loss of approximately 80 percent of the crop. The fungus

was considered to belong to the *Pythium gracile* group, but the species was not identified. While studying the pathogenicity of various pythiaceous isolates obtained mainly from pineapple, Sideris (59) claimed that maize was susceptible to *Nematosporangium* spp. The occurrence of the *Pythium* root rot disease of maize and sugar cane in the Philippine Islands was reported by Roldan (55), who stated that young maize plants as well as those approaching maturity died because of root rot. Koehler and Holbert (34) in 1930 claimed that in soils of well-balanced fertility, *Pythium* was the only fungus that had been definitely determined as causing a rot of the adventitious root system. The influence of temperature on the pathogenicity of strains of *Pythium* isolated from the infected roots of sugar cane and inoculated on maize was discussed by Flor (15). A further report on *Pythium* root-rot disease of maize in the Philippine Islands was given in 1932 by Roldan (56), who described the fungus and named it *Pythium arrhenomanes* var. *philippinensis*.

The causal agent of browning root rot of wheat in Canada was described and named by Vanterpool and Truscott (70) as *Pythium arrhenomanes* var. *canadensis*. They reported that the fungus was severely destructive to maize, wheat and rye. *Pythium epigynum*, a new species named by Hohnk (25), was reported capable of attacking young maize seedlings.

Various strains of *Pythium arrhenomanes* isolated from infected roots of sugar cane and maize from various parts of the United States, Canada, Hawaii and India were studied extensively by Rands and Dopp (53) in 1934. Data obtained from the comparison of morphological characters, cultural studies and infection experiments revealed that *P. arrhenomanes* was composed of a considerable number of variously differentiated forms. Sideris (60) in his work on the pythiaceous diseases of tropical plants included maize as one of the hosts of *Nematosporangium aphanidermatum*. A species of *Pythium* causing root rot and collapse of very young seedlings of sugar cane was reported by Subramaniam (65) in 1936 from India. Crop plants such as wheat, oats and barley were actively parasitized by the fungus in cross-inoculation tests, maize and sorghum being mildly attacked. The distinct characteristics that could be used to separate *P. graminicola* from *P. arrhenomanes* were described and illustrated by Drechsler (11). In 1938 Ho and Melhus (21) claimed that strains of *P. graminicola* obtained from the

infected roots of barley were highly pathogenic to maize seedlings.

Pythium graminicola was isolated frequently from the roots of plants obtained from fields in Iowa. Only occasionally was the organism isolated from decayed seed, most of the seed decay having been caused by *P. debaryanum*. Typical symptoms are shown in fig. 2.

There was no doubt about the pathogenicity of this organism on maize, but the injury caused under different environmental conditions was not well known. To determine the type and extent of injury experiments were conducted in the laboratory, greenhouse and field using different infested and noninfested soils.

The soil used in the laboratory experiments was a naturally infested Webster silty loam with a pH 6.2 to 6.6 obtained at Kanawha. The seed used was inbred Lancaster 317. The germination of the seeds in the infested soil was about 49 percent lower at 15° C. than at 26° C., but the germination in the check pots (steamed soil) showed no marked difference at either temperature.

Isolations made from the infected seeds yielded *P. graminicola*, *P. debaryanum*, *Gibberella saubinetii*, *Trichoderma lignorum*, *Fusarium moniliforme*, and *Fusarium* spp. Brown, water-soaked necrotic lesions were observed on the root tips of the plants taken from the infested soil. The lesions were more distinct and abundant on the roots of the plants taken from the 26° chamber than on those from the 15° chamber. All isolates obtained from the infected root tips were *P. graminicola*. It seemed that this pathogen caused more seed decay at low than at high temperatures and that root infection was favored by high temperatures.

The injury caused by *P. graminicola* on maize was studied further with artificially infested steamed soil in the greenhouse. The soil used was Clarion loam with a pH 6.2 to 6.6 and was taken from the experimental plots at Ames. Seeds of inbred Lancaster 317, Black 345 and hybrid Iowa 939 were employed.

The percentage decrease of stand count of inbred Lancaster 317 was about three times as great in set B as in set A, and the decrease of root length in set B was twice that in set A (table 3). The percentage decrease of top growth also was greater in set B than in set A.

The response of inbred Black 345 to *P. graminicola* (table 4) was, except for top growth, very similar to that of Lancaster 317. The decrease of root length of both inbreds in



Fig. 2. Characteristic symptoms of *Pythium graminicola* on maize seedlings, inbred Lancaster 317, grown in the greenhouse. A. The tip of the primary root, of two adventitious roots and of several rootlets discolored and rotted. Isolated necrotic lesions also on the remaining portions of three adventitious roots. Note the discolored pericarp of the seed. B. The three seminal roots completely discolored and rotted. Necrotic lesions on the tips and remaining portions of several adventitious roots. C. The tips of three young adventitious roots discolored and rotted. Lesions on the primary root and young rootlets.

TABLE 3. AVERAGE STAND, TOP GROWTH AND ROOT LENGTH (CM.) OF MAIZE SEEDLINGS GROWN FROM 10 SEEDS OF INBRED LANCASTER 317 IN SOIL INFESTED OR NOT INFESTED WITH *PYTHIUM GRAMINICOLA* AT TWO TEMPERATURES IN GREENHOUSE EXPERIMENTS.

Temperature	20°-28° C. (A)				9° and 20°-28° C. (B)				Mean difference at two temperatures
Series	1	2	3	Mean	1	2	3	Mean	
Stand count									
Soil not infested.....	10.0	9.0	8.5	9.2	9.0	8.5	8.0	8.5	
Soil infested.....	8.0	8.0	7.0	7.7	5.0	4.5	5.0	3.8	
Decrease.....	2.0	1.0	1.5	1.5	4.0	4.0	3.0	3.7	2.2
Percentage decrease.....				16.4				43.5	
Value of t.....				5.2*				10.9†	6.1*
Top growth									
Soil not infested.....	24	23	30	25.7	29	29	30	29.3	
Soil infested.....	19	17	22	19.3	20	18	17	18.3	
Decrease.....	5	6	8	6.4	9	11	13	11.0	4.6
Percentage decrease.....				24.9				37.5	
Value of t.....				7.2*				9.6*	13.7†
Root length									
Soil not infested.....	6.0	6.5	6.0	6.2	6.0	4.5	5.5	5.3	
Soil infested.....	4.0	5.0	5.0	4.7	3.0	2.0	3.0	2.6	
Decrease.....	2.0	1.5	1.0	1.5	3.0	2.5	2.5	2.7	1.2
Percentage decrease.....				24.1				50.9	
Value of t.....				5.2*				15.7†	6.9*

* Between 5 percent and 1 percent levels.

† Beyond 1 percent level.

set B over that in set A (tables 3 and 4) might be due primarily to the retarding effect of low temperature on the development of the seedlings during the first 10 days of the experiment. Though the roots in set B were shorter, the necrotic lesions occurring on them were decidedly smaller and less abundant than those on roots in set A.

With hybrid Iowa 939 the percentage decreases of stand count, top growth, and root length of the seedlings grown in the infested soil were low and not significant (table 5). Both inbreds Black 345 and Lancaster 317 were highly susceptible to *P. graminicola*, but hybrid Iowa 939 showed little injury.

Field trials were conducted at Ames and at Conesville in 1939 and 1940. Seed of inbred Lancaster 317 was used. At Ames the percentage decreases of stand count, top growth and root length in 1939 and 1940 were very similar (table 6). The type and extent of injury in these field experiments corresponded closely with those obtained in the greenhouse experiments. At Conesville the injuries were not statistically significant (table 7). The small amount of injury to the

TABLE 4. AVERAGE STAND, TOP GROWTH AND ROOT LENGTH (CM.) OF MAIZE SEEDLINGS GROWN FROM 10 SEEDS OF INBRED BLACK 345 IN SOIL INFESTED OR NOT INFESTED WITH *PYTHIUM GRAMINICOLA* AT TWO TEMPERATURES IN GREENHOUSE EXPERIMENTS.

Temperature	20°-28° C. (A)				9° and 20°-28° C. (B)				Mean difference at two temperatures
Series	1	2	3	Mean	1	2	3	Mean	
Stand count									
Soil not infested.....	9.0	7.5	8.0	8.2	9.0	7.5	9.0	8.5	
Soil infested.....	5.0	5.0	6.0	5.3	3.0	2.0	5.0	3.3	
Decrease.....	4.0	2.5	2.0	2.9	6.0	5.5	4.0	5.2	2.3
Percentage decrease.....				34.8				60.8	
Value of t.....				4.7*				8.6*	7.0*
Top growth									
Soil not infested.....	21	24	23	22.7	12	20	22	18.0	
Soil infested.....	16	15	17	16.0	7	12	16	11.7	
Decrease.....	5	9	6	6.7	5	8	6	6.3	-0.4
Percentage decrease.....				29.5				35.0	
Value of t.....				5.5*				7.2*	1.0
Root length									
Soil not infested.....	5.5	6.0	5.5	5.7	5.0	6.0	5.5	5.5	
Soil infested.....	3.5	5.0	4.0	4.2	2.0	3.0	1.5	2.2	
Decrease.....	2.0	1.0	1.5	1.5	3.0	3.0	4.0	3.3	1.8
Percentage decrease.....				26.5				60.6	
Value of t.....				5.2*				10.0†	4.2*

* Between 5 percent and 1 percent levels.

† Beyond 1 percent level.

TABLE 5. AVERAGE STAND, TOP GROWTH AND ROOT LENGTH (CM.) OF MAIZE SEEDLINGS GROWN FROM 10 SEEDS OF HYBRID IOWA 939 IN SOIL INFESTED OR NOT INFESTED WITH *PYTHIUM GRAMINICOLA* AT TWO TEMPERATURES IN GREENHOUSE EXPERIMENTS.

Temperature	20°-28° C. (A)				9° and 20°-28° C. (B)				Mean difference at two temperatures
Series	1	2	3	Mean	1	2	3	Mean	
Stand count									
Soil not infested.....	10.0	9.5	10.0	9.8	9.5	10.0	10.0	9.8	
Soil infested.....	8.5	9.5	10.0	9.3	8.0	10.0	9.5	9.2	
Decrease.....	1.5	0	0	.5	1.5	0	.5	.6	.1
Percentage decrease.....				5.1				6.7	
Value of t.....				1.0				1.5	0.2
Top growth									
Soil not infested.....	38	24	26	29.6	26	22	29	25.7	
Soil infested.....	29	23	25	26.0	16	18	28	20.7	
Decrease.....	9	1	1	3.6	10	4	1	5.0	1.4
Percentage decrease.....				12.3				19.5	
Value of t.....				1.4				1.9	0.4
Root length									
Soil not infested.....	9.5	6.5	6.5	7.5	8.0	7.0	6.0	7.0	
Soil infested.....	8.0	6.5	6.0	6.8	6.5	6.0	6.0	6.2	
Decrease.....	1.5	0	.5	.7	1.5	1.0	0	.8	
Percentage decrease.....				9.3				11.4	
Value of t.....				1.5				1.9	0.3

TABLE 6. AVERAGE STAND, TOP GROWTH AND ROOT LENGTH (CM.) OF MAIZE SEEDLINGS GROWN FROM 50 SEEDS OF INBRED LANCASTER 317 IN SOIL INFESTED OR NOT INFESTED WITH *PYTHIUM GRAMINICOLA* IN EARLY (MIDDLE OF APRIL) FIELD PLANTINGS AT AMES.

Year	1939				1940				Mean 2 years
Replication	1	2	3	Mean	1	2	3	Mean	
Stand count									
Soil not infested.....	45	49	46	47.6	45	49	47	47.0	46.8
Soil infested.....	42	44	41	42.3	41	43	42	42.0	42.2
Decrease.....	3	5	5	4.3	4	6	5	5.0	4.7
Percentage decrease.....				9.2				10.6	9.9
Value of t.....				6.4*				8.7*	11.1†
Top growth									
Soil not infested.....	32	34	35	33.7	38	41	40	39.7	36.7
Soil infested.....	28	27	26	27.0	32	31	30	31.0	29.0
Decrease.....	4	7	9	6.7	6	10	10	8.7	7.7
Percentage decrease.....				19.8				20.9	20.4
Value of t.....				4.6*				6.5*	7.7†
Root length									
Soil not infested.....	6.5	7.0	7.0	6.8	6.5	8.0	7.5	7.3	7.1
Soil infested.....	4.0	5.0	5.0	4.6	4.5	5.5	5.0	5.0	4.8
Decrease.....	2.5	2.0	2.0	2.2	2.0	2.5	2.5	2.3	2.3
Percentage decrease.....				31.6				31.8	31.7
Value of t.....				13.0†				14.0†	20.3†

* Between 5 percent and 1 percent levels.

† Beyond 1 percent level.

TABLE 7. AVERAGE STAND, TOP GROWTH AND ROOT LENGTH (CM.) OF MAIZE SEEDLINGS GROWN FROM 50 SEEDS OF INBRED LANCASTER 317 IN SOIL INFESTED OR NOT INFESTED WITH *PYTHIUM GRAMINICOLA* IN EARLY (MIDDLE OF APRIL) FIELD PLANTINGS AT CONESVILLE.

Year	1939				1940				Mean 2 years
Replication	1	2	3	Mean	1	2	3	Mean	
Stand count									
Soil not infested.....	40	43	44	42.3	42	46	41	43.0	42.7
Soil infested.....	40	42	40	40.6	40	42	42	41.3	41.0
Decrease.....	0	1	4	1.7	2	4	-1	1.7	1.7
Percentage decrease.....				4.0				3.9	4.0
Value of t.....				1.4				1.2	2.0
Top growth									
Soil not infested.....	30	35	37	34.0	40	39	35	38.0	36.0
Soil infested.....	31	30	32	31.0	38	40	39	39.0	35.0
Decrease.....	-1	5	5	3.0	2	-1	-4	-1.0	1.0
Percentage decrease.....				8.8				-2.6	2.8
Value of t.....				1.5				-0.6	0.7
Root length									
Soil not infested.....	6.5	7.5	7.0	7.0	7.0	7.5	6.5	7.0	7.0
Soil infested.....	7.0	7.0	6.5	6.8	7.0	7.5	6.0	6.8	6.8
Decrease.....	-5	5	5	2	0	0	5	2	2
Percentage decrease.....				2.9				2.9	2.9
Value of t.....				0.5				1.0	1.0

germinating seeds and seedlings was thought to be due to the relatively high temperature (20° C.) when the plantings were made. Temperature specifically influences the amount of infection and the subsequent development of the disease caused by *P. graminicola*. This statement is fully substantiated by the data shown in tables 3, 4 and 5.

GIBBERELLA SAUBINETII (MONT.) SACC.

The importance of *Gibberella saubinetii* in relation to the root rot of maize was investigated by Hoffer and Holbert (23), Hoffer, Johnson and Atanasoff (24) and others (8, 14, 49, 67, 69). Recently Goddard (16) in her study on variation in *G. saubinetii* suggested that the saltation in this organism was somatic rather than germinal.

Gibberella saubinetii was the organism most commonly isolated from the necrotic lesions on roots and mesocotyls of maize seedlings and was observed frequently on the pericarp of decayed seed. It frequently was found associated with or following injury by *Pythium graminicola*. Though more than 600 isolates of this organism were obtained from plants grown at Kanawha, Ames and Conesville, only a few of them were injurious to maize. Symptoms caused by *G. saubinetii* are illustrated in fig. 3.

Experiments were conducted in the greenhouse and field to measure the injuries caused by a virulent strain of this organism. Seed of inbred Lancaster 317 was used.

The greenhouse experiments were carried out on artificially infested steamed Clarion loam. In set B the percentage decrease of stand count and top growth was nearly twice that in set A, and the percentage decrease of root length was about one-third more than that of set A (table 8). While only one of the differences is statistically significant, they are all in the same direction and indicate that this strain of *G. saubinetii* was more destructive to maize under conditions promoting slow germination and seedling development (set B) than under conditions favorable for more rapid growth (set A).

Field experiments were conducted at Ames in 1939 and 1940 in soil that was mainly a Clarion loam. The injuries sustained by the seedlings in the field trials were similar to but not so severe as those manifested in the greenhouse, as may be noted by comparing tables 8 and 9.

HELMINTHOSPORIUM SATIVUM P., K. and B.

Diseases of maize caused by *Helminthosporium* probably were noticed first as a leaf blight by Passerini (48) in Italy,

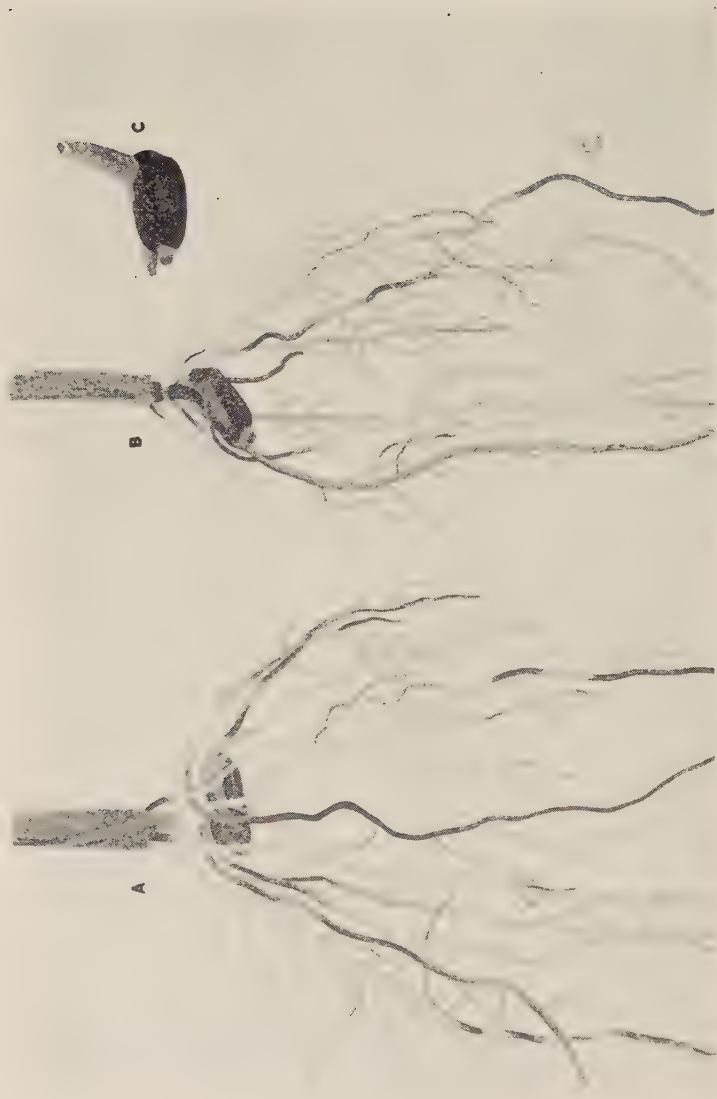


Fig. 3. Characteristic symptoms of *Gibberella saubinetii* on maize seedlings, inbred Lancaster 317, grown in the greenhouse. A. Brown necrotic lesions on various parts of the primary, seminal and adventitious roots and rootlets. Two of the seminal roots discolored and rotted. The pericarp of the seed dark brown. B. Lesions on various parts of the seminal and adventitious roots. Most of the rootlets of the primary root destroyed. Note the dark discolored and rotted mesocotyl. The pericarp of the seed was deep red. C. Seedling blight with the radicle and plumule discolored and rotted. The pericarp was deep red.

TABLE 8. AVERAGE STAND, TOP GROWTH AND ROOT LENGTH (CM.) OF MAIZE SEEDLINGS GROWN FROM 10 SEEDS OF INBRED LANCASTER 317 IN SOIL INFESTED OR NOT INFESTED WITH *GIBBERELLA SAUBINETII* (VIRULENT STRAIN 4052) AT TWO TEMPERATURES IN GREENHOUSE EXPERIMENTS.

Temperature	20°-28° C. (A)				9° and 20°-28° C. (B)				Mean difference at two temperatures
eries	1	2	3	Mean	1	2	3	Mean	
Stand count									
Soil not infested.....	9.5	9.0	10.0	9.5	8.5	9.0	9.0	8.8	
Soil infested.....	7.5	6.0	9.0	7.5	5.5	5.0	6.0	5.5	
Decrease.....	2.0	3.0	1.0	2.0	3.0	4.0	3.0	3.3	1.3
Percentage decrease.....				21.5				39.9	
Value of t.....				3.5				10.0†	4.0
Top growth									
Soil not infested.....	29	24	32	28.3	26	29	28	27.7	
Soil infested.....	22	18	28	22.7	15	17	20	17.3	
Decrease.....	7	6	4	5.6	11	12	8	10.4	4.8
Percentage decrease.....				20.0				37.4	
Value of t.....				6.5*				8.6*	7.0*
Root length									
Soil not infested.....	8.0	7.0	6.5	7.2	8.0	9.0	8.0	8.3	
Soil infested.....	5.0	4.5	4.5	4.7	4.0	4.5	4.0	4.2	
Decrease.....	3.0	2.5	2.0	2.5	4.0	4.5	4.0	4.1	1.6
Percentage decrease.....				34.7				50.0	
Value of t.....				9.0*				25.1†	5.0

* Between 5 percent and 1 percent levels.

† Beyond 1 percent level.

who named the fungus *Helminthosporium turcicum*. Peck (50) reported, as early as 1881, a maize disease that he attributed to a species of *Helminthosporium* and called the fungus *H. inconspicuum*. In 1922 Stover (63) noted that an unidentified species of *Helminthosporium* caused a marked seedling blight and rotting of the mesocotyl, cotyledonary node and seminal roots of the plants, but he gave no detailed description of the symptoms. Seedling blight was very marked at temperatures ranging from 16° to 24° C., especially at 20° C. Drechsler (9) decided that *H. inconspicuum* was more closely related to *H. sativum* than to *H. turcicum*. Mitra (44) made an intensive study of *H. turcicum* and concluded that: "The fungus is found on leaves only, and forms long, narrow spots of dirty brown or straw color."

Helminthosporium turcicum was never isolated from the roots, but *H. sativum* occurred regularly on the roots and mesocotyls of maize seedlings from various parts of Iowa throughout the entire growing season. *Helminthosporium sativum* occasionally was associated with seed decay, but in

TABLE 9. AVERAGE STAND, TOP GROWTH AND ROOT LENGTH (CM.) OF MAIZE SEEDLINGS GROWN FROM 50 SEEDS OF INBRED LANCASTER 317 IN SOIL INFESTED OR NOT INFESTED WITH *GIBBERELLA SAUBINETII* (VIRULENT STRAIN 4052) IN EARLY (MIDDLE OF APRIL) FIELD PLANTINGS AT AMES.

Year	1939				1940				Mean 2 years
Replication	1	2	3	Mean	1	2	3	Mean	
Stand count									
Soil not infested.....	49	47	46	47.3	49	47	47	47.7	47.5
Soil infested.....	43	42	38	41.0	40	39	41	40.0	40.5
Decrease.....	6	5	8	6.3	9	8	6	7.7	7.0
Percentage decrease.....				13.3				16.1	14.7
Value of t.....				7.2*				7.2*	11.1†
Top growth									
Soil not infested.....	35	30	36	33.7	42	40	40	40.7	37.2
Soil infested.....	28	25	27	26.7	32	30	33	31.7	29.2
Decrease.....	7	5	9	7.0	10	10	7	9.0	8.0
Percentage decrease.....				20.8				22.1	21.4
Value of t.....				6.1*				9.0*	9.8†
Root length									
Soil not infested.....	8.0	9.0	8.5	8.5	12.0	10.5	11.0	11.2	9.9
Soil infested.....	6.0	6.5	7.0	6.5	8.5	8.5	7.5	8.2	7.4
Decrease.....	2.0	2.5	1.5	2.0	3.5	2.0	3.5	3.0	2.5
Percentage decrease.....				23.5				26.8	25.2
Value of t.....				6.9*				6.0*	7.3†

* Between 5 percent and 1 percent levels.

† Beyond 1 percent level.

most cases it was a secondary invader on decayed seeds previously parasitized by *Pythium debaryanum*, *P. graminicola* or *Gibberella saubinetii*. Inoculation experiments indicated that the majority of the isolates of this organism were non-virulent, but in the late summer of 1939 eight isolates that were pathogenic in greenhouse trials were obtained from badly diseased hybrid maize in a field near Waukeg, Iowa. Characteristic symptoms produced by this organism are illustrated in fig. 4.

The injuries caused by two strains (6002 and 9035) of *H. sativum* were measured and compared in the greenhouse experiments described below. The injuries caused by the latter strain were studied further under field conditions. Seed of inbred Lancaster 317 was used in both experiments.

Infection experiments in the greenhouse were carried out in Clarion loam. Strain 9035 of *H. sativum* was highly virulent on maize, and its virulence increased, though not significantly, when the plants were subjected to low temperatures (table 10). Strain 6002 apparently was only

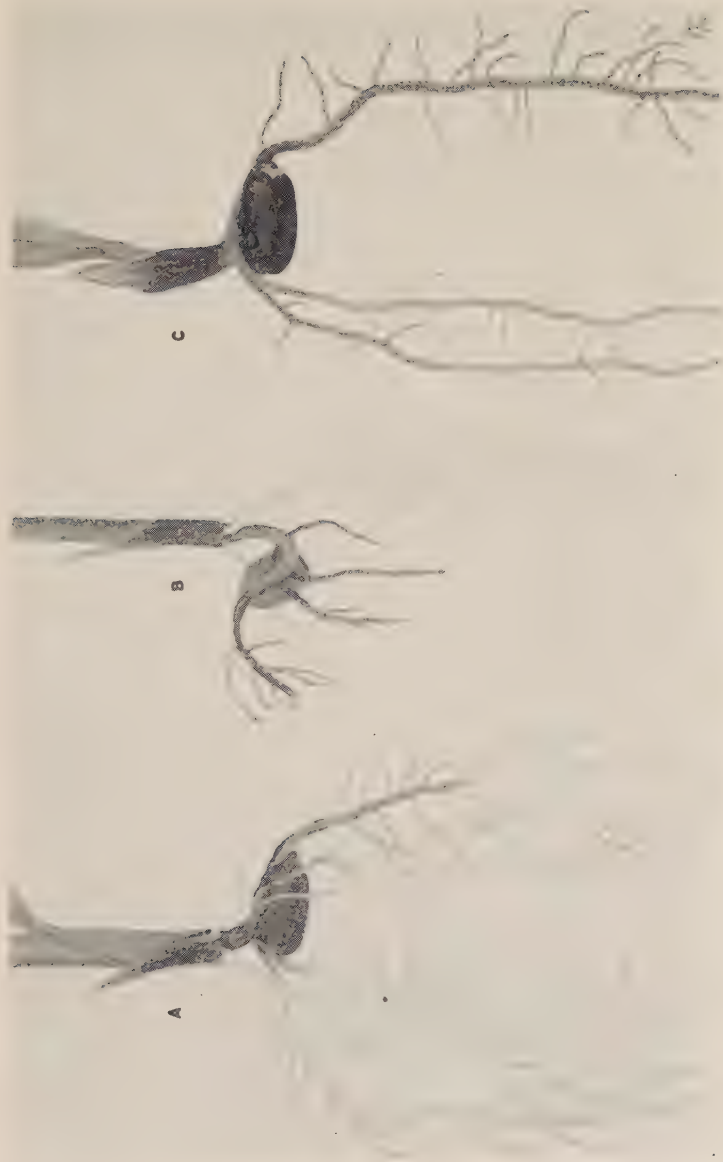


Fig. 4. Characteristic symptoms of *Helminthosporium sativum* on maize seedlings, inbred Lancaster 317, grown in the greenhouse. A. Necrotic lesions on the basal portions of the primary and seminal roots. The mesocotyl discolored and rotted, and the pericarp of the seed and basal sheath brownish black. B. The primary root, three young seminal roots and the mesocotyl discolored and rotted. The pericarp of the seed and basal sheath brownish black. C. All the roots and the mesocotyl discolored and rotted. The pericarp of the seed and basal sheath black.

TABLE 10. AVERAGE STAND, TOP GROWTH AND ROOT LENGTH (CM.) OF MAIZE SEEDLINGS GROWN FROM 10 SEEDS OF INBRED LANCASTER 317 IN SOIL INFESTED OR NOT INFESTED WITH *HELMINTHOSPORIUM SATIVUM* (HIGHLY VIRULENT STRAIN 9035) AT TWO TEMPERATURES IN GREENHOUSE EXPERIMENTS.

Temperature	20°-28° C. (A)				9° and 20°-28° C. (B)				Mean difference at two temperatures
Series	1	2	3	Mean	1	2	3	Mean	
Stand count									
Soil not infested	8.5	9.0	9.5	9.0	8.5	10.0	9.5	9.3	
Soil infested	5.0	7.0	6.5	6.2	4.0	5.5	3.5	4.3	
Decrease	3.5	2.0	3.0	2.8	4.5	4.5	6.0	5.0	2.2
Percentage decrease				31.4				53.6	
Value of t				6.4*				10.0†	3.6
Top growth									
Soil not infested	32	30	30	30.7	28	28	30	28.7	
Soil infested	25	26	23	24.7	19	18	16	17.7	
Decrease	7	4	7	6.0	9	10	14	11.0	5.0
Percentage decrease				19.5				38.3	
Value of t				6.0*				7.2*	3.3
Root length									
Soil not infested	6.5	7.0	6.5	6.7	5.5	6.5	6.0	6.0	
Soil infested	4.5	4.5	5.0	4.7	3.0	3.5	2.0	2.8	
Decrease	2.0	2.5	1.5	2.0	2.5	3.0	4.0	3.2	1.2
Percentage decrease				30.0				52.8	
Value of t				6.9*				7.2*	1.8

* Between 5 percent and 1 percent levels.

† Beyond 1 percent level.

slightly virulent on maize (table 11). No pronounced influence of temperature was shown.

Field studies of the injuries caused by strain 9035 were conducted at Ames and Kanawha in 1940. The percentage decreases of stand, top growth and root length at Ames and at Kanawha agreed closely with each other (table 12). The type of injury produced in the field was similar to that observed in the greenhouse tests, except that the stand decrease was less in the field experiments.

RHIZOCTONIA SOLANI KÜHN

The first observation of *Rhizoctonia solani* on maize probably was reported by Rolfs (57) from Florida in 1905. In 1915 Peltier (51) published an extensive account of parasitic *Rhizoctonia* in America, in which he mentioned that *R. solani* had been isolated from maize roots in Illinois. An undetermined species of *Rhizoctonia* was isolated from root lesions on maize plants in the field by Valteau (68) in Kentucky. Matz (41) in 1921 claimed that *R. palida* was found on roots of young maize seedlings in Puerto Rico in association with a root decay and chlorosis of the plants. A sclero-

TABLE 11. AVERAGE STAND, TOP GROWTH AND ROOT LENGTH (CM.) OF MAIZE SEEDLINGS GROWN FROM 10 SEEDS OF INBRED LANCASTER 317 IN SOIL INFESTED OR NOT INFESTED WITH *HELMINTHOSPORIUM SATIVUM* (SLIGHTLY VIRULENT STRAIN 6002) AT TWO TEMPERATURES IN GREENHOUSE EXPERIMENTS.

Temperature	20°-28° C. (A)				9° and 20°-28° C. (B)				Mean difference at two temperatures
Series	1	2	3	Mean	1	2	3	Mean	
Stand count									
Soil not infested.....	9.0	9.5	10.0	9.5	8.0	9.5	9.0	8.8	
Soil infested.....	8.0	9.0	8.0	8.3	7.0	9.0	7.0	7.6	
Decrease.....	1.0	.5	2.0	1.2	1.0	.5	2.0	1.2	0
Percentage decrease.....				12.6				13.6	
Value of t.....				2.6				2.6	0
Top growth									
Soil not infested.....	27	24	32	27.7	22	29	28	26.3	
Soil infested.....	25	23	28	25.4	20	29	26	25.0	
Decrease.....	2	1	4	2.3	2	0	2	1.3	1.0
Percentage decrease.....				8.3				4.9	
Value of t.....				2.6				2.0	0.9
Root length									
Soil not infested.....	6	5	5.5	5.5	5.0	5.5	6.5	5.7	
Soil infested.....	6	5	5.0	5.3	4.0	5.5	6.0	5.2	
Decrease.....	0	0	.5	.2	1.0	0	.5	.5	.3
Percentage decrease.....				3.1				8.8	
Value of t.....				1.0				1.7	1.0

TABLE 12. AVERAGE STAND, TOP GROWTH AND ROOT LENGTH (CM.) OF MAIZE SEEDLINGS GROWN FROM 50 SEEDS OF INBRED LANCASTER 317 IN SOIL INFESTED OR NOT INFESTED WITH *HELMINTHOSPORIUM SATIVUM* (HIGHLY VIRULENT STRAIN 9035) IN EARLY FIELD PLANTINGS AT AMES (MIDDLE OF APRIL) AND KANAWHA (LAST WEEK OF APRIL) IN 1940.

Location	Ames				Kanawha				Mean two locations
Replication	1	2	3	Mean	1	2	3	Mean	
Stand count									
Soil not infested.....	49	49	47	48.3	49	47	46	47.3	47.8
Soil infested.....	44	45	44	44.3	46	45	42	44.3	44.3
Decrease.....	5	4	3	4.0	3	2	4	3.0	3.5
Percentage decrease.....				8.3				6.3	7.3
Value of t.....				6.9*				5.2*	8.2†
Top growth									
Soil not infested.....	29	35	32	32.0	41	38	39	39.3	35.6
Soil infested.....	25	28	25	26.0	36	33	36	35.0	30.5
Decrease.....	4	7	7	6.0	5	5	3	4.3	5.1
Percentage decrease.....				18.8				10.9	14.8
Value of t.....				6.0*				6.4*	7.9†
Root length									
Soil not infested.....	6.5	7.0	8.0	7.2	7.0	6.5	7.5	7.0	7.1
Soil infested.....	5.0	5.5	6.0	5.5	5.5	4.5	6.5	5.5	5.5
Decrease.....	1.5	1.5	2.0	1.7	1.5	2.0	1.0	1.5	1.6
Percentage decrease.....				23.3				21.4	22.4
Value of t.....				10.0†				5.2*	10.3†

* Between 5 percent and 1 percent levels.

† Beyond 1 percent level.

tial disease of maize caused by *R. solani* was reported by Bertus (1) from Ceylon.

Rayllo (54) reported from Russia that sclerotia of *Hypochnus* (*Rhizoctonia solani*) were found on the roots of maize. A strain of *R. solani* with large sclerotia was found on maize by Deighton (6) in Sierra Leone. Mackie (38) observed a charcoal rot of maize and beans in California and assumed that the causal organism was *R. bataticola*. The affected plants showed signs of premature ripening and later might bend or break just above the crown, causing severe lodging. Small black sclerotia were found abundantly in the stalk and roots. *Rhizoctonia solani* was reported recently by Samuel and Garrett (58) and Hynes (27) as the cause of a severe root rot of wheat, barley and oats in Australia.

In 1934 *R. zeae*, a new species, was described by Voorhees (71) as causing a sclerotial rot of maize in Florida. The fungus caused seed decay and was capable of rotting the seedling roots and mesocotyls but was most important in causing an ear rot. Ho and Melhus (22) claimed that *R. solani* was one of the severe root parasites of maize seedlings. They reported that the fungus often appeared on the mesocotyl and later caused cortical necrosis of the roots.

Rhizoctonia solani was isolated frequently from the necrotic lesions on roots and mesocotyls of maize seedlings at Kanawha. A high percentage of isolates of this organism was obtained also from the decorticated roots of maize at Conesville in 1938 and at Ames in 1939. Typical symptoms are shown in fig. 5.

The following experiments using virulent strain 747 and seed of inbred Lancaster 317 were performed in the greenhouse and under field conditions as a means for estimating the injuries caused by this organism. In greenhouse experiments strain 747 decreased the percentage of germination, reduced the top growth and injured the roots (table 13). The injury to germination was greater where the seed was started at 8° C. (set B) than when started at 20° to 28° C. (set A), but the difference was not statistically significant. In field trials using the same strain of *Rhizoctonia* for 2 years, 1939 and 1940, (table 14) the type of injury was the same, but it was less severe in the field than in the greenhouse trials.

DIPLODIA ZEAE (SCHW.) LÉV.

The parasitism of *Diplodia zeae* on maize was first recorded by Heald (18) in 1906. The nomenclature, life history

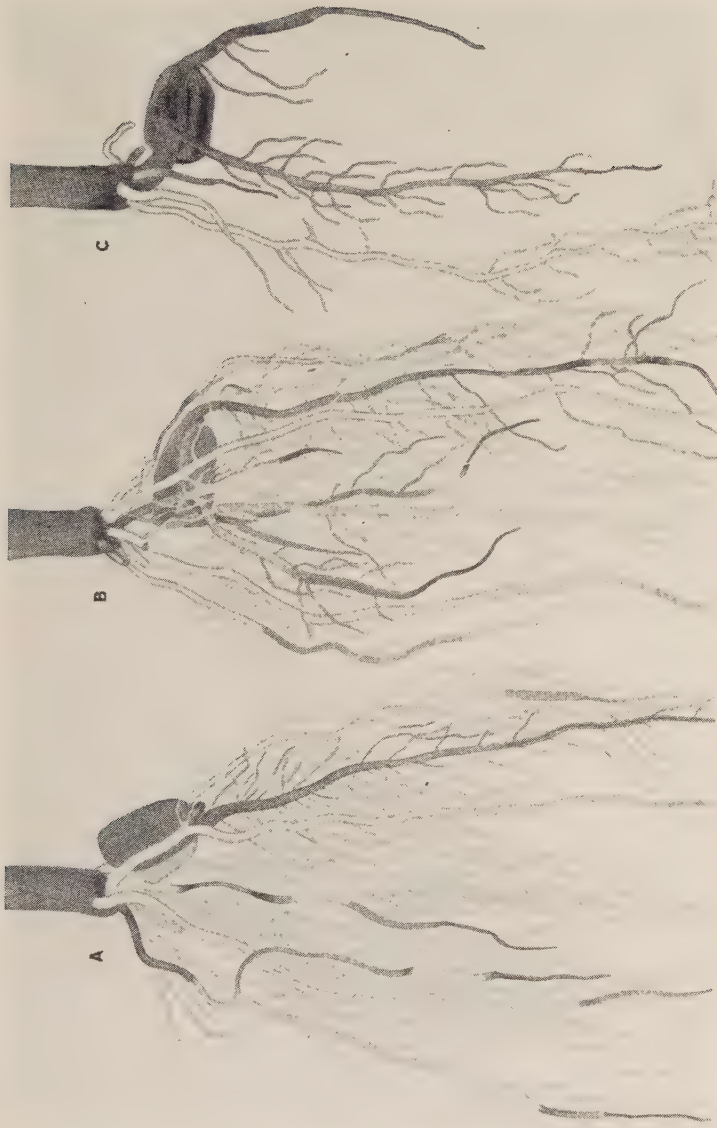


Fig. 5. Characteristic symptoms of *Rhizoctonia solani* on maize seedlings, inbred Lancaster 317, grown in the greenhouse. A. Decorticated necrotic lesions on various parts of the seminal and adventitious roots. The primary root completely discolored and rotted. The pericarp of the seed brown. B. Decorticated necrotic lesions on various parts of the primary, seminal and adventitious roots. The basal portion of the mesocotyl discolored and rotted. C. Decorticated necrotic lesions on various parts of the primary seminal and two young adventitious roots. The entire mesocotyl discolored and rotted.

TABLE 13. AVERAGE STAND, TOP GROWTH AND ROOT LENGTH (CM.) OF MAIZE SEEDLINGS GROWN FROM 10 SEEDS OF INBRED LANCASTER 317 IN SOIL INFESTED OR NOT INFESTED WITH *RHIZOCTONIA SOLANI* (VIRULENT STRAIN 747) AT TWO TEMPERATURES IN GREENHOUSE EXPERIMENTS.

Temperature	20°-25° C. (A)				9° and 20°-28° C. (B)				Mean difference at two temperatures
Series	1	2	3	Mean	1	2	3	Mean	
Stand count									
Soil not infested.....	9.0	8.5	8.0	8.5	8.0	9.0	7.5	8.2	
Soil infested.....	7.5	6.0	7.0	6.8	6.0	5.0	6.0	5.7	
Decrease.....	1.5	2.5	1.0	1.7	2.0	4.0	1.5	2.5	.9
Percentage decrease.....				19.7				30.6	
Value of t.....				3.8				3.3	2.5
Top growth									
Soil not infested.....	29	24	23	25.3	22	29	29	26.7	
Soil infested.....	22	11	8	13.7	20	18	24	20.7	
Decrease.....	7	13	15	11.6	2	11	5	6.0	-5.6
Percentage decrease.....				45.9				22.5	
Value of t.....				4.9*				2.3	2.4
Root length									
Soil not infested.....	8.0	7.5	7.5	7.7	6.0	7.0	8.0	7.0	
Soil infested.....	4.0	1.5	2.0	2.5	3.5	3.5	3.5	3.5	
Decrease.....	4.0	6.0	5.5	5.2	2.5	3.5	4.5	3.5	-1.7
Percentage decrease.....				67.4				50.0	
Value of t.....				8.6*				6.1*	3.8

*Between 5 percent and 1 percent levels.

and parasitism of this organism were discussed further by Heald, Wilcox and Pool (19) and Burrill and Barrett (3). *Diplodia* was found fruiting on the roots, crown, and at the base of the stem of maize by Smith and Hedges (61). The mycelium of the organism was found in abundance in the interior of the root and stem. They assumed that the manner of infection of this organism was from the soil into the roots, from these to the interior of the stems and thence upward to the cobs, and finally to the kernels. Melhus and Durrell (42) doubted that *D. zeae* traveled from the soil up to the ears inside the stalk although they admitted that the fungus attacked the roots. The extensive reviews of literature made by Clayton (4), Raleigh (52) and McNew (40) preclude further mention in this bulletin of previous contributions.

In greenhouse trials to determine the injury caused by *D. zeae* little decrease in the stand occurred at high temperatures, but this did not mean that the organism caused no injury to the plants. There was a 41.8 percent decrease in root length and over 16 percent decrease in top growth

TABLE 14. AVERAGE STAND, TOP GROWTH AND ROOT LENGTH (CM.) OF MAIZE SEEDLINGS GROWN FROM 50 SEEDS OF INBRED LANCASTER 317 IN SOIL INFESTED OR NOT INFESTED WITH *RHIZOCTONIA SOLANI* (VIRULENT STRAIN 747) IN EARLY (MIDDLE OF APRIL) FIELD PLANTINGS AT AMES.

Year	1939				1940				Mean two years
Replication	1	2	3	Mean	1	2	3	Mean	
Stand count									
Soil not infested.....	46	48	49	47.6	47	46	48	47.0	47.3
Soil infested.....	42	45	43	43.4	44	42	43	43.0	43.2
Decrease.....	4	3	6	4.3	3	4	5	4.0	4.1
Percentage decrease.....				9.0				8.5	8.8
Value of t.....				4.9*				6.9*	8.7†
Top growth									
Soil not infested.....	35	29	33	32.3	40	42	39	40.3	36.3
Soil infested.....	30	27	29	28.7	35	36	36	35.7	32.2
Decrease.....	5	2	4	3.6	5	6	3	4.6	4.1
Percentage decrease.....				11.2				10.7	10.9
Value of t.....				4.2*				5.3*	6.9†
Root length									
Soil not infested.....	8.0	8.0	9.0	8.3	7.0	8.0	9.0	8.0	8.2
Soil infested.....	6.5	7.0	7.5	7.0	5.0	6.5	7.5	6.3	6.6
Decrease.....	1.5	1.0	1.5	1.3	2.0	1.5	1.5	1.7	1.5
Percentage decrease.....				15.7				21.3	18.5
Value of t.....				8.0*				10.0†	11.6†

* Between 5 percent and 1 percent levels.

† Beyond 1 percent level.

(table 15). In other words, *Diplodia* may cause seedling injury at high temperatures even though the number of plants that emerge is not reduced significantly. In the cultures started at low temperature, 9° C., the injury to germination became pronounced, resulting in a decrease of over 44 percent.

Symptoms observed in these trials are shown in fig. 6.

PENICILLIUM OXALICUM CURRIE AND THOM

The injury caused by *Penicillium* was not clarified until 1928, when Johann (28) described some of the characteristic symptoms of this disease. Later Johann (29) claimed that *Penicillium oxalicum* was not aggressive enough to enter vigorous, living cells but produced a toxic substance that might injure or kill the cells in advance of the organism. In 1930 Koehler and Holbert (34) mentioned that seed inoculations with pure cultures of *P. oxalicum* caused striking reductions in stand and vigor as compared with check plots grown from similar but uninoculated seed. Further studies

TABLE 15. AVERAGE STAND, TOP GROWTH AND ROOT LENGTH (CM.) OF MAIZE SEEDLINGS GROWN FROM 10 SEEDS OF INBRED LANCASTER 317 IN SOIL INFESTED OR NOT INFESTED WITH *DIPLODIA ZEAE* AT TWO TEMPERATURES IN GREENHOUSE EXPERIMENTS.

Temperature	20°-28° C. (A)				9° and 20°-28° C. (B)				Mean difference at two temperatures
Series	1	2	3	Mean	1	2	3	Mean	
Stand count									
Soil not infested.....	9.0	9.5	10.0	9.5	8.5	9.0	9.5	9.0	
Soil infested.....	8.0	9.0	10.0	9.0	4.0	4.0	7.0	5.0	
Decrease.....	1.0	0.5	0	.5	4.5	5.0	2.5	4.0	3.5
Percentage decrease.....				5.3				44.4	
Value of t.....				1.7				5.3*	4.3*
Top growth									
Soil not infested.....	29	24	32	28.3	22	29	28	26.3	
Soil infested.....	21	19	31	23.6	20	19	21	20.0	
Decrease.....	8	5	1	4.7	2	10	7	6.3	1.6
Percentage decrease.....				16.6				24.0	
Value of t.....				2.3				2.7	0.5
Root length									
Soil not infested.....	8.0	7.0	6.5	7.2	6.5	9.0	7.0	7.5	
Soil infested.....	5.0	3.0	4.5	4.2	4.5	4.0	4.0	4.2	
Decrease.....	3.0	4.0	2.0	3.0	2.0	5.0	3.0	3.3	0.3
Percentage decrease.....				41.8				44.4	
Value of t.....				5.2*				3.8	0.3

* Between 5 percent and 1 percent levels.

on *Penicillium* injury to maize were reported by Johann, Holbert, and Dickson (32), who found that the characteristic symptoms were yellowing of the leaves, retardation of the growth and, in cases of severe infection, more or less complete drying of the leaves. The dead plants, both in the greenhouse and in the field, remained upright. Diachun (7) recently reported that severe injury and stunting of maize seedlings were observed when the kernels were wounded and inoculated by immersion in a spore suspension or were wounded and planted in soil infested with *P. oxalicum*.

During the past four growing seasons *P. oxalicum* was one of the most common organisms isolated from maize. Although it occurred frequently on decayed seeds and infected tissue of the underground parts, other virulent pathogens such as *Pythium debaryanum*, *P. graminicola*, *Gibberella saubinetii*, etc., were found to have invaded these parts previous to the appearance of *Penicillium oxalicum*. This organism, therefore, was considered to be one of the secondary invading organisms.

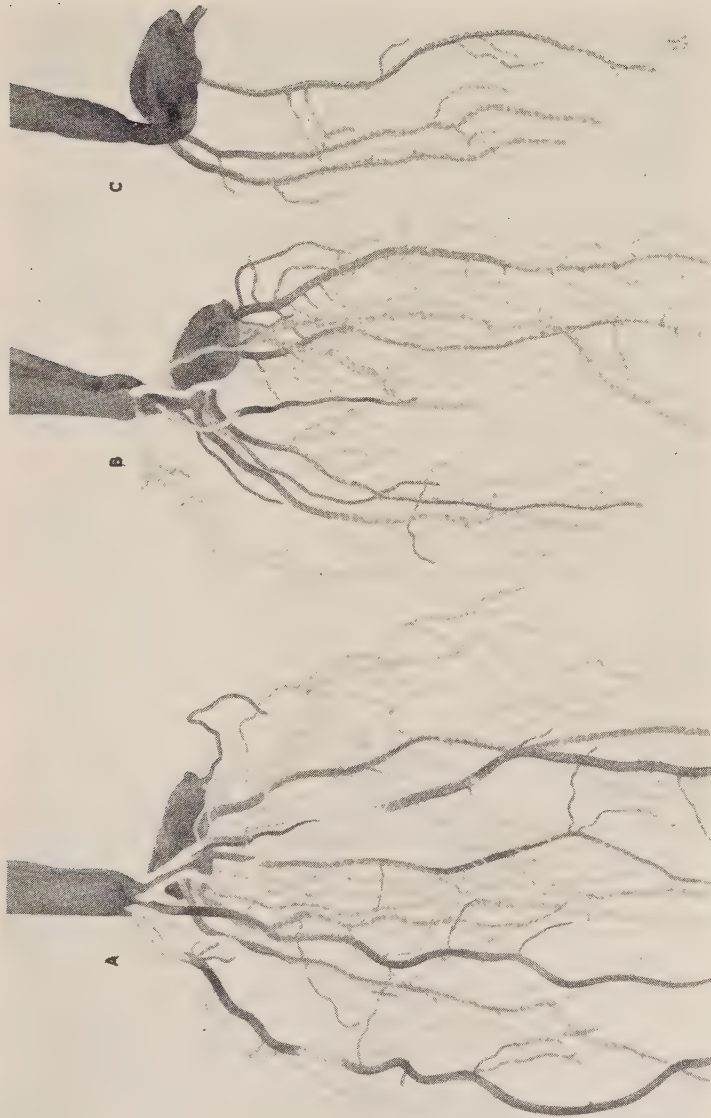


Fig. 6. Characteristic symptoms of *Diplodia zeae* on maize seedlings, variety inbred Lancaster 317, grown in the greenhouse. A. Necrotic lesions on various parts of the primary, seminal and adventitious roots. The basal portion of the mesocotyl discolored and rotted. B. The mesocotyl and primary root completely discolored and rotted. C. Seedling with all the young roots rotted. The pericarp of the seed was dark brown.

TABLE 16. AVERAGE STAND, TOP GROWTH AND ROOT LENGTH (CM.) OF MAIZE SEEDLINGS GROWN FROM 10 SEEDS OF INBRED LANCASTER 317 IN SOIL INFESTED OR NOT INFESTED WITH *PENICILLIUM OXALICUM* AT TWO TEMPERATURES IN GREENHOUSE EXPERIMENTS.

Temperature	20°-28° C. (A)				9° and 20°-28° C. (B)				Mean difference at two temperatures
Series	1	2	3	Mean	1	2	3	Mean	
Stand count									
Soil not infested.....	8.0	10.0	8.0	8.7	7.0	9.5	8.0	8.2	
Soil infested.....	7.0	9.0	5.0	7.0	6.0	9.0	6.0	7.0	
Decrease.....	1.0	1.0	3.0	1.7	1.0	0.5	2.0	1.2	-5
Percentage decrease.....				19.3				14.3	
Value of t.....				2.5				2.6	0.6
Top growth									
Soil not infested.....	23	32	36	30.3	29	29	35	31.0	
Soil infested.....	22	28	31	27.0	18	25	30	27.7	
Decrease.....	1	4	5	3.3	1	4	5	3.3	0
Percentage decrease.....				10.9				10.7	
Value of t.....				2.8				2.8	0
Root length									
Soil not infested.....	7.5	6.0	8.0	7.2	8.0	6.5	7.5	7.3	
Soil infested.....	7.0	6.0	7.0	6.7	7.0	5.5	6.0	6.2	
Decrease.....	1.5	0	1.0	.5	1.0	1.0	1.5	1.1	0.7
Percentage decrease.....				7.0				16.0	
Value of t.....				1.7				7.0*	2.0

* Between 5 percent and 1 percent levels.

To estimate the injuries caused by *P. oxalicum* experiments were performed in the greenhouse. Seed of inbred Lancaster 317 was planted.

From the data in table 16 it is apparent that *P. oxalicum* caused a root necrosis at the low temperature. Although some seed decay occurred at low and high temperatures, the stand differences were not statistically significant. The effect on top growth was not pronounced after the initial stages of germination and emergence of the seedling.

FUSARIUM MONILIFORME SHELTON

A serious root and stalk disease of maize caused by a species of *Fusarium* in Iowa was reported by Pammel (46) in 1914. Two years later Pammel, King and Seal (47) published their studies on the root, stalk, and ear rot of maize caused by a species of *Fusarium*. Although they were unable to show the connection between this fungus and *Gibberella saubinetii*, the symptoms described were similar to those caused by the latter organism. Numerous isolations

of *Fusarium moniliforme* from rotting roots and stems of maize seedlings were made by Valleau (68) in Kentucky, who was inclined to believe that *F. moniliforme* was the primary cause of root and stalk rots of maize in that state as well as in the other central and southern states.

Holbert et al. (26) in their field inoculation studies of root, stalk, and ear rot diseases of maize, failed to obtain definite data concerning the pathogenicity of *F. moniliforme* as a root rot parasite of maize. They suggested that several strains of this organism existed and that undoubtedly they differed greatly in their ability to parasitize the maize plant. In 1926 Valleau, Karraker and Johnson (69) concluded from their study on maize root rot that *F. moniliforme* was a semiparasitic organism that appeared to destroy the temporary root system but caused very little injury other than occasional rotting of a small lateral.

The symptoms of and the temperature effect of *F. moniliforme* on maize were described by Limber (37) who stated that: "The characteristic lesions produced by *F. moniliforme* on the mesocotyl are small, oval, yellowish areas, with darker spots in the center in old lesions. On the roots the lesions are usually darker. At a temperature of 24° C. small rotted areas may be produced on the mesocotyl, usually close to the seed. At temperatures above 28° C. a serious injury may occur." In 1932 the pathogenicity and variability of *F. moniliforme* were investigated thoroughly by Leonian (36) who found that the organism was very unstable and dissociated into many forms. Even the most vigorous strains exhibited variability in their pathogenicity. Successful inoculations were accomplished largely through wounds and at low temperatures ranging from 20° to 23° C. Voorhees (72) suggested that the invasions of the mesocotyl resulted from direct penetration of the epidermis, or through ruptures in the cortex produced by the emergence of adventitious roots. The fungus also might enter the primary radicle directly through the epidermis, but it usually entered through ruptures produced in the cortex by the emergence of lateral roots.

Fusarium moniliforme was isolated frequently from the decayed seeds, infected roots and mesocotyls of seedlings collected from various parts of Iowa. It was isolated also from the crowns of older plants. Isolation studies indicated that this organism occurred on maize throughout the entire growing season. The results of the present study, however, indicate that *F. moniliforme* is a secondary invading or-

TABLE 17. AVERAGE STAND, TOP GROWTH AND ROOT LENGTH (CM.) OF MAIZE SEEDLINGS GROWN FROM 10 SEEDS OF INBRED LANCASTER 317 IN SOIL INFESTED OR NOT INFESTED WITH *FUSARIUM MONILIFORME* AT TWO TEMPERATURES IN GREENHOUSE EXPERIMENTS.

Temperature	20°-28° C. (A)				9° and 20°-28° C. (B)				Mean difference at two temperatures
Series	1	2	3	Mean	1	2	3	Mean	
Stand count									
Soil not infested.....	9.0	9.0	8.5	8.8	8.0	9.0	10.0	9.0	
Soil infested.....	10.0	7.0	8.0	8.3	8.0	6.0	9.0	7.7	
Decrease.....	-1.0	2.0	.5	.5	0	3.0	1.0	1.3	.8
Percentage decrease.....				5.7				14.8	
Value of t.....				0.6				1.5	0.7
Top growth									
Soil not infested.....	29	24	30	27.7	22	29	30	27.0	
Soil infested.....	29	22	29	26.7	22	24	28	24.7	
Decrease.....	0	2	1	1.0	0	5	2	2.3	1.3
Percentage decrease.....				3.6				8.5	
Value of t.....				1.7				1.6	0.9
Root length									
Soil not infested.....	8.0	7.0	7.5	7.5	6.0	8.0	7.5	7.2	
Soil infested.....	8.0	6.5	7.5	7.3	6.5	5.0	6.0	5.8	
Decrease.....	0	.5	0	.2	-.5	3.0	1.5	1.3	1.2
Percentage decrease.....				2.3				18.7	
Value of t.....				1.0				1.3	1.1

ganism, appearing on decayed seeds, infected roots, mesocotyls and crowns of the plants.

Experiments to estimate accurately the injury caused by *F. moniliforme* under controlled conditions were conducted in the greenhouse. Data taken from plants in set A at 5 weeks and from the plants in set B at 6 weeks are summarized in table 17. Since all differences were small and non-significant, the strains of *F. moniliforme* isolated from the infected roots and mesocotyls of maize were believed to be only slightly virulent and chiefly saprophytic in habit.

ASPERGILLUS NIGER VAN TIEG.

In 1920 Taubenhaus (66) demonstrated that rotting of the roots of seedlings could be obtained by planting seeds from ears infected with *Aspergillus niger* or by adding the spores of this organism to the soils in which the seedlings were grown. Koehler (33) mentioned that *Rhizopus* and *Aspergillus* were isolated frequently from the mesocotyls of infected plants grown in soil following seed inoculation with these organisms. In a later publication, Koehler and Holbert (34) reported that inoculations with *A. niger* pro-

duced a pronounced rot of the embryo region and mesocotyl. The fungus invaded the inner tissue of the mesocotyl, presumably gaining entrance at the base of the primary roots or through the scutellum. Recently, seedling invasion and mesocotyl rot caused by *A. flavus* and *A. tamarii* were studied by Koehler and Woodworth (35).

Since *A. niger* was reported as attacking the mesocotyls and roots and was observed so generally on maize in the field, it seemed desirable to study its destructiveness on maize under controlled conditions in the greenhouse. Seed of inbred Lancaster 317 was sown in artificially infested steamed Clarion loam.

Data were taken from the plants in set A after 5 weeks and from plants in set B after 6 weeks. As indicated in table 18, the decrease of stand count, top growth and root length of plants grown in the infested soil in set A were slight. There was a slight decrease of stand count and root length in set B, but in neither case was the decrease significant. The decrease of top growth in set B was inconsistent. These facts suggested that *A. niger* was not an active pathogen and apparently occurred as a secondary invader on decayed seeds and infected mesocotyls or roots.

TABLE 18. AVERAGE STAND, TOP GROWTH AND ROOT LENGTH (CM.) OF MAIZE SEEDLINGS GROWN FROM 10 SEEDS OF INBRED LANCASTER 317 IN SOIL INFESTED OR NOT INFESTED WITH *ASPERGILLUS NIGER* AT TWO TEMPERATURES IN GREENHOUSE EXPERIMENTS.

Temperature	20°-28° C. (A)				9° and 20°-28° C. (P)				Mean difference at two temperatures
Series	1	2	3	Mean	1	2	3	Mean	
Stand count									
Soil not infested.....	8.0	10.0	8.0	8.7	8.0	9.5	9.0	8.8	
Soil infested.....	9.0	10.0	8.0	9.0	8.0	9.0	8.0	8.3	
Decrease.....	-1.0	0	0	-3	0	.5	1.0	.5	
Percentage decrease.....								5.7	
Value of t.....				-1.0				1.7	1.9
Top growth									
Soil not infested.....	23	30	36	29.7	29	32	35	32.0	
Soil infested.....	20	36	34	30.0	30	29	33	30.7	
Decrease.....	3	-6	2	-.3	-1	3	2	1.3	
Percentage decrease.....								4.1	
Value of t.....				-0.1				1.1	0.5
Root length									
Soil not infested.....	7.5	6.0	8.0	7.2	8.0	6.5	8.0	7.5	
Soil infested.....	7.5	6.5	7.0	7.0	7.5	6.0	8.0	7.2	
Decrease.....	0	-.5	1.0	.2	.5	.5	0	.3	.1
Percentage decrease.....				2.4				4.4	
Value of t.....				0.4				2.0	0.3

TABLE 19. SUMMARY OF SYMPTOMS PRODUCED BY *Pythium debaryanum*, *Pythium graminicola*, *Gibberella saubinetii*, *Helminthosporium sativum*, *Rhizoctonia solani*, *Diplodia zeae* AND *Penicillium oxalicum* ON SEEDS AND SEEDLINGS OF MAIZE IN GREENHOUSE EXPERIMENTS, USING INBRED LANCASTER 317.

Pathogen	Symptoms (Seedlings were subjected to the temperature and moisture conditions favorable for infection.)		
	On seeds	On aboveground parts	On mesocotyl and roots
<i>Pythium debaryanum</i>	Decay severe; seeds coated with soil particles and hyphae; water-soaking and bluish discoloration of pericarp; internal structure rotted.	Stunting; yellowing and curling of basal leaves.	Brownish water-soaked lesions confined chiefly to root tips. No mesocotyl rot.
<i>Pythium graminicola</i>	Decay moderate; seeds coated with soil particles and hyphae; water-soaking and bluish discoloration of pericarp; internal structure rotted.	Stunting; seedling blight; yellowing, rolling and drying of basal leaves.	Large, brownish, water-soaked lesions on the roots. Few lesions on the mesocotyl. Frequently rootlets destroyed. Oospores or filamentous sporangia often present in tissue.
<i>Gibberella saubinetii</i>	Decay moderate; reddish brown to deep red discoloration of pericarp; seed coated with hyphae and soil particles; internal structure rotted.	Stunting; seedling blight; yellowing, drooping and drying of basal leaves.	Reddish brown to dark red discoloration and rot of roots, mesocotyl and plumule. Mesocotyl rot chiefly at points where secondary roots emerged.
<i>Helminthosporium sativum</i>	Decay moderate; seed coated with hyphae and soil particles; bluish black discoloration of pericarp; internal structure rotted.	Stunting; seedling blight; yellowing and drying of basal leaves.	Brownish or bluish black discoloration, shrivelling and rot of roots, mesocotyl and basal portion of stalk.
<i>Rhizoctonia solani</i>	Decay slight, seed coated with hyphae and soil particles; pericarp dark brown; internal structure rotted.	Stunting; seedling blight; yellowing, curling and drying of basal leaves.	Light to dark brown lesions on roots, mesocotyl and basal sheath; roots decorticated in some areas.
<i>Diplodia zeae</i>	Decay moderate; bluish discoloration of pericarp; internal structure rotted.	Pronounced stunting; seedling blight; yellowing and drying of basal leaves.	Brown discoloration and shrivelling of mesocotyl; brown lesions on roots and crowns.
<i>Penicillium oxalicum</i>	Seed decay slight; shrivelling; internal structure rotted; sporulation on pericarp.	Stunting; seedling blight; yellowing, drooping and drying of basal leaves.	Dark brown necrotic lesions on roots and mesocotyls.

THE SYMPTOMS AND INJURIES RESULTING WHEN TWO ORGANISMS ATTACK THE PLANT AT THE SAME TIME

Since maize grown in the field is subject to attack not only by one organism but also by two or more pathogens simultaneously, greenhouse studies of the effect of two organisms attacking the host at the same time seemed worthwhile.

Many different trials were made using two pathogens. The characteristic response obtained is shown in tables 20, 21 and 22. The same technique was used in these studies as was employed in the studies with one organism. Records were accumulated on the injury to stand, top and root growth at two temperature conditions in two different lots of seed, the open pollinated variety Kossuth County Reliance and the inbred Lancaster 317.

Some method for evaluating the disease severity was necessary for the comparison of injuries resulting from various combinations of pathogens. The method used in each case was a modification of the method described by McKinney (39) and later modified by Greaney and Machacek (17). The different classes of injury and their numerical evaluation together with a formula for arriving at a numerical value for the disease severity follow.

Class	Type of injury occurring on individual plant	Evaluation
1	Healthy.	0
2	Small necrotic lesions on root tips; leaves healthy or slightly yellow.	1
3	Distinct necrotic lesions on root tips and remaining portions of roots; leaves markedly yellow.	2
4	Most of root tips destroyed and remaining portions of roots covered with numerous necrotic lesions; leaves curled and dried.	3
5	Seed decay or seedling blight.	4

$$\text{Disease severity} = \frac{\text{The sum of the evaluation of the different classes} \times 100}{\text{Number of seeds planted} \times 4}$$

In general the injury to the maize plants was greater at the low-high alternating (8° and 22°-27° C.) temperature than at the constantly high (22°-27° C.) temperature. This difference was particularly marked on the stand and in the total severity evaluation. In all combinations of *P. debaryanum* and *P. graminicola* with *G. saubinetii*, *H. sativum* and *R. solani* the disease severity was greater than the severity

TABLE 20. THE INJURY INDUCED WHEN TWO ACTIVE PATHOGENS WERE ALLOWED TO ATTACK SIMULTANEOUSLY MAIZE SEEDLINGS GROWING IN THE GREENHOUSE AT TWO TEMPERATURES. INBRED LANCASTER 317 WAS USED UNLESS OTHERWISE INDICATED.

Combination of pathogens	Stand count		Top growth (cm.)		Root length (cm.)		Severity evaluation	
	High ^a temp.	Low ^b temp.	High temp.	Low temp.	High temp.	Low temp.	High temp.	Low temp.
Check ^c	10	9	28	30	12	12	0	0
<i>P. debaryanum</i> ^c	9	3	32	22	13	10	10	68
<i>P. debaryanum</i> <i>G. saubinetii</i>	7	3	26	22	9	7	54	72
<i>P. debaryanum</i> <i>H. sativum</i>	8	2	23	20	8	6	50	80
<i>P. debaryanum</i> ^c <i>D. zae</i>	9	2	28	19	11	8	20	85
<i>P. debaryanum</i> ^c <i>R. solani</i>	6	1	25	20	8	8	50	90
<i>P. debaryanum</i> ^c <i>P. graminicola</i>	6	1	18	16	3	4	85	92
<i>P. graminicola</i>	8	6	22	20	4	3	60	65
<i>P. graminicola</i> <i>R. solani</i>	6	5	22	18	4	6	62	70
<i>P. graminicola</i> <i>H. sativum</i>	7	3	19	16	5	3	64	76
<i>P. graminicola</i> <i>D. zae</i>	8	5	17	14	4	5	66	78
<i>P. graminicola</i> <i>G. saubinetii</i>	8	5	15	13	5	4	64	70

^a Plants held at temperatures ranging from 22° to 27° C.

^b Plants held at 8° C. for first 10 days, then at 22° to 27° C.

^c Seedlings of variety Kossuth County Reliance.

of any of the corresponding pathogens when they occurred alone (tables 20 and 21). The combination of the two species of *Pythium* caused greater injury than any other combination. In some cases the symptoms of one organism were predominant because the conditions that prevailed were unfavorable for the other organism; *P. graminicola* is such an example. When *P. debaryanum* was combined with *H. sativum*, *D. zae*, *R. solani*, or *P. graminicola* at high temperatures, the symptoms of *P. debaryanum* were not so pronounced as those of the other pathogens, but at low temperatures the symptoms of each pathogen were distinct. In combinations of *P. graminicola* with *R. solani*, *H. sativum*, *D. zae* or *G. saubinetii* at high temperatures, on the other hand, the symptoms of each pathogen were distinct, but at low temperatures those of *P. graminicola* were most preva-

TABLE 21. THE INJURY INDUCED WHEN TWO ACTIVE PATHOGENS WERE ALLOWED TO ATTACK SIMULTANEOUSLY MAIZE SEEDLINGS GROWING IN THE GREENHOUSE AT TWO TEMPERATURES. INBRED LANCASTER 317.

Combination of pathogens	Stand count		Top growth (cm.)		Root length (cm.)		Severity evaluation	
	High ^a temp.	Low ^b temp.	High temp.	Low temp.	High temp.	Low temp.	High temp.	Low temp.
<i>G. saubinetii</i>	8	6	25	20	10	7	51	63
<i>G. saubinetii</i> <i>R. solani</i>	9	5	20	15	7	4	42	70
<i>G. saubinetii</i> <i>D. zeae</i>	8	3	24	12	9	4	42	76
<i>G. saubinetii</i> <i>H. sativum</i>	8	4	23	26	9	6	42	72
<i>H. sativum</i>	7	5	22	20	8	6	45	67
<i>H. sativum</i> <i>D. zeae</i>	6	5	20	21	8	7	56	62
<i>H. sativum</i> <i>R. solani</i>	8	7	18	23	5	9	55	45
<i>R. solani</i>	7	6	17	24	6	8	46	50
<i>R. solani</i> <i>D. zeae</i>	7	5	17	22	6	8	48	60

^a Plants held at temperatures ranging from 22° to 27°C.

^b Plants held at 8°C. for first 10 days, then at 22° to 27°C.

lent. This behavior of the two species of *Pythium* is in line with their growth response to high and low temperature. The combination of *G. saubinetii* with *R. solani* or *H. sativum* at low temperatures tended to increase the disease severity over that of any of these three pathogens occurring alone (table 21). In no combination was the severity increased beyond the sum of the severity index of the two pathogens associated except in the combination of *P. debaryanum* with *P. graminicola* at high temperature. *Penicillium oxalicum* and *A. niger* either showed only a slight tendency to increase the injury or seemed to have a depressing influence.

TABLE 22. THE INJURY INDUCED WHEN AN ACTIVE AND A SECONDARY PATHOGEN WERE ALLOWED TO ATTACK SIMULTANEOUSLY MAIZE SEEDLINGS GROWING IN THE GREENHOUSE AT TWO TEMPERATURES. INBRED LANCASTER 317 USED UNLESS OTHERWISE INDICATED.

Combination of pathogens	Stand count		Top growth (cm.)		Root length (cm.)		Severity evaluation	
	High ^a temp.	Low ^b temp.	High temp.	Low temp.	High temp.	Low temp.	High temp.	Low temp.
<i>P. ozalicum</i> ^c								
<i>P. debaryanum</i>	6	2	30	18	10	8	47	80
<i>P. ozalicum</i>								
<i>P. graminicola</i>	8	6	21	22	9	7	60	70
<i>P. ozalicum</i>								
<i>G. saubinetii</i>	8	5	25	23	9	8	27	65
<i>P. ozalicum</i>								
<i>H. sativum</i>	8	2	23	20	8	6	50	80
<i>P. ozalicum</i>								
<i>R. solani</i>	9	7	22	25	8	7	22	35
<i>A. niger</i> ^c								
<i>P. debaryanum</i>	9	1	32	30	12	9	12	81
<i>A. niger</i>								
<i>P. graminicola</i>	8	5	20	18	9	7	45	65
<i>A. niger</i>								
<i>G. saubinetii</i>	9	3	26	22	10	6	35	75
<i>A. niger</i>								
<i>H. sativum</i>	9	5	18	16	6	4	34	60
<i>A. niger</i>								
<i>R. solani</i>	9	9	23	26	12	12	20	12

^aPlants held at temperatures ranging from 22° to 27°C.

^bPlants held at 8°C. for first 10 days, then at 22° to 27°C.

^cSeedlings of variety Kossuth County Reliance.

THE SUCCESSION OF SOIL-INHABITING PLANT PATHOGENS ON THE SEEDLINGS OF MAIZE UNDER FIELD CONDITIONS

Following greenhouse studies of two soil-inhabiting pathogens attacking the host simultaneously, attention was turned to the condition in the field. A determination of the succession of these pathogens in the field was established through isolations made at one- and two-week intervals until the third week of June from decayed seeds and from plants grown from seed of inbred Lancaster 317 and inbred Black 345 at Kanawha in 1938, 1939 and 1940. The number of isolations varied from year to year and from interval to interval.

Pythium debaryanum was the most prevalent active pathogen isolated from the decayed seeds in May (table 23). *Gibberella saubinetii* was the only other active organism found on the seeds. Other pathogens isolated from the seeds were *Penicillium oxalicum*, *Trichoderma lignorum* and *Fusarium* spp. The two species of *Pythium* seemed to predominate on the roots during the last part of May and the first of June. Less prevalent pathogens on the young seedlings were *G. saubinetii*, *Rhizoctonia solani*, *Helminthosporium sativum*, *T. lignorum*, *Fusarium moniliforme* and *Fusarium* spp. *Pythium graminicola* remained active in the roots in the third week of June, *P. debaryanum* having disappeared. Other organisms isolated at this time were *T. lignorum*, *Fusarium* spp., *G. saubinetii*, *R. solani*, *Aspergillus niger*, *H. sativum* and *F. moniliforme*.

A definite succession of pathogens was found on the roots beginning with the third week in May and extending into the third week of June. *Pythium debaryanum* was first on the germinated seed and was followed on the seedling in June by *P. graminicola* and various other organisms cited in table 23.

TABLE 23. OCCURRENCE OF SOIL-INHABITING PATHOGENS ON DECAYED SEEDS, INFESTED ROOTS, MESOCOTYLS AND PLUMULES OF SEEDLINGS OF INBRED LANCASTER 317 IN 1938 AND 1939 AND INBRED BLACK 345 IN 1940 AT KANAWHA.

Date	1938				1939				1940			
	Part of host	No. isolates	Percent isolates	Organism	Part of host	No. isolates	Percent isolates	Organism	Part of host	No. isolates	Percent isolates	Organism
Third week of May	Seeds	55	72	<i>P. debaryanum</i>					Seeds	42	49	<i>P. debaryanum</i>
	"	5	6	<i>G. saubinetii</i>					"	15	18	<i>G. saubinetii</i>
	"	7	9	<i>T. lignorum</i>					"	26	33	<i>Fusarium</i> spp.
	"	10	13	<i>Fusarium</i> spp.					Root tips	34	75	<i>P. debaryanum</i>
									Mesocotyls	11	25	<i>Fusarium</i> spp.
									"	32	29	<i>G. saubinetii</i>
									"	36	34	<i>Fusarium</i> spp.
									"	30	28	<i>R. solani</i>
									"	9	8	<i>Rhizopus</i> sp.
									Plumules	46	71	<i>G. saubinetii</i>
									"	21	21	<i>Fusarium</i> spp.
									"	5	8	<i>Rhizopus</i> sp.
Fourth week of May	Roots	92	37	<i>P. debaryanum</i>	Seeds	34	52	<i>P. debaryanum</i>	Roots	21	8	<i>P. debaryanum</i>
	"	13	5	<i>P. graminicola</i>	"	16	24	<i>G. saubinetii</i>	"	79	32	<i>P. graminicola</i>
	"	37	15	<i>G. saubinetii</i>	"	3	5	<i>P. ozalium</i>	"	63	25	<i>G. saubinetii</i>
	"	18	7	<i>R. solani</i>	"	5	8	<i>T. lignorum</i>	"	87	35	<i>Fusarium</i> spp.
	"	13	5	<i>H. sativum</i>	"	7	11	<i>Fusarium</i> spp.	Mesocotyls	27	50	<i>R. solani</i>
	"	15	6	<i>T. lignorum</i>					"	12	22	<i>P. graminicola</i>
First week of June	"	12	5	<i>F. moniliforme</i>					"	15	28	<i>G. saubinetii</i>
	"	50	20	<i>Fusarium</i> spp.					Plumules	24	75	<i>G. saubinetii</i>
									"	8	25	<i>Fusarium</i> spp.
	Roots	28	16	<i>P. debaryanum</i>	Roots	44	35	<i>P. graminicola</i>	Seeds	18	40	<i>T. lignorum</i>
	"	52	29	<i>P. graminicola</i>	"	12	10	<i>P. debaryanum</i>	"	17	39	<i>Fusarium</i> spp.
	"	7	4	<i>G. saubinetii</i>	"	15	12	<i>G. saubinetii</i>	"	9	21	<i>P. ozalium</i>
	"	16	9	<i>H. sativum</i>	"	11	9	<i>R. solani</i>	Mesocotyls	41	56	<i>G. saubinetii</i>
	"	25	10	<i>R. solani</i>	"	7	6	<i>H. sativum</i>	"	6	8	<i>R. solani</i>
	"	18	14	<i>T. lignorum</i>	"	14	11	<i>T. lignorum</i>	"	20	27	<i>T. lignorum</i>
	"	6	3	<i>F. moniliforme</i>	"	5	4	<i>F. moniliforme</i>	"	7	9	<i>Fusarium</i> spp.
Third week of June	"	22	12	<i>Fusarium</i> spp.								
	"	6	3	<i>Monospora</i> sp.								
	Roots	34	28	<i>P. graminicola</i>	Roots	78	22	<i>P. graminicola</i>	Root tips	5	10	<i>P. graminicola</i>
	"	17	14	<i>G. saubinetii</i>	"	29	8	<i>G. saubinetii</i>	"	5	9	<i>G. saubinetii</i>
	"	18	15	<i>R. solani</i>	meso-cotyls	25	7	<i>R. solani</i>	"	19	35	<i>T. lignorum</i>
	"	7	6	<i>H. sativum</i>	"	18	5	<i>H. sativum</i>	"	8	15	<i>A. niger</i>
	"	10	9	<i>T. lignorum</i>	"	36	10	<i>P. ozalium</i>	"	17	31	<i>Fusarium</i> spp.
	"	10	9	<i>F. moniliforme</i>	"	65	18	<i>T. lignorum</i>	Mesocotyls	7	10	<i>G. saubinetii</i>
	"	23	19	<i>Fusarium</i> spp.	"	108	30	<i>Fusarium</i> spp.	"	8	10	<i>R. solani</i>
									"	42	60	<i>T. lignorum</i>
									"	14	20	<i>Fusarium</i> spp.

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